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Vertical distribution of spiders (Araneae) in Central European shallow subterranean habitats

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Abstract

Shallow subterranean habitats are among the last habitats in Central Europe to be arachnologically researched. Using stratified pipe traps, we studied the vertical distribution of spiders in soil and interspaces in bedrock (shallow subterranean habitats). Specifically, we sampled fauna in different substrates, including limestone, sandy marlstone, sandy marl, claystone, loess, and artificial gravel accumulation. Employing stratified pipe traps allowed us to identify the depth at which particular species occurred. Across multiple years and sampling sites, we collected 76 spider species, 21 of which showed an affinity for subterranean microhabitats. Some of these species occurred in interspaces in soil and bedrock, whereas others have been previously found in subterranean ant nests and animal burrows. We collected five species (*Iberina microphthalma*, *Centromerus* cf. *piccolo*, *Porrhomma cambridgei*, *P. microcavense*, and *P. microps*) almost exclusively at depths over half a meter, suggesting the strong affinity of these species for a subterranean lifestyle. We provide diagrams of these species' vertical distribution and photo-document eye reduction. Our study demonstrates that poorly studied shallow subterranean habitats harbor diverse subterranean spider fauna, including several previously considered rare species in Central Europe.

Keywords

Araneae, *Centromerus*, Czechia, edaphomorphism, *Iberina*, micropthalmic spiders, *Palliduphantes*, pipe traps, *Porrhomma*, soil spiders, troglomorphism

Introduction

Subterranean habitats range from large spaces in caves to tiny spaces such as fissure networks in bedrock or soil pores (Culver and Pipan 2014). They are typically characterized by the absence of light, almost 100% relative air humidity, and small temperature fluctuations over a year (Badino 2010). Animals fully adapted to subterranean habitats, e.g., those demonstrating depigmentation, reduced eyes, and other so-called troglomorphic traits, are sometimes caught by pitfall traps also on the surface but usually as singletons and only during the humid and cold season (Czech Arachnological Society 2022). Some subterranean habitats in central Europe are already well-studied from an arachnological point of view, especially caves or scree slopes (Růžička et al. 1995; Růžička et al. 2012). Caves and scree slopes provide relatively large spaces and thus can be inhabited by troglomorphic spiders like *Bathyphantes eumenis buchari* Růžička, 1988 (Růžička 1988) and *Meta menardi* (Latreille, 1804) (Mammola and Isaia 2014). In contrast, the arachnofauna of shallow subterranean habitats within coarse substrata, which are difficult to access, is poorly studied (Mammola et al. 2016). Shallow subterranean habitats are areas of habitable space that are less than 10 m in depth from the surface. These range from large areas such as shallow caves and lava tubes, to tiny areas such as cracks in ceilings, or spaces in soil (Culver and Pipan 2014). Different authors have used perforated pipe traps with one cup on the bottom to sample invertebrates in shallow subterranean habitats and deep soil strata (e.g., López and Oromí 2010; Deltshev et al. 2011; Jiménez-Valverde et al. 2015; Mammola et al. 2017; Ledesma et al. 2020). Conversely, Schlick-Steiner and Steiner (2000) constructed a trap consisting of a perforated pipe and a set of removable plastic cups situated on a central metal axis. The cups collect animals entering the pipe through holes at different depths, allowing to study the vertical distribution of arthropods in shallow subterranean habitats. For example, this trap design has been used to study the vertical distribution of spiders in soil and fissured rock (Laška et al. 2011; Růžička and Dolanský 2016), and springtails (Rendoš et al. 2016; Jureková et al. 2021), and myriapods (Haľková et al. 2020) in forested scree slopes.

In this study, we employed stratified pipe traps to explore spider fauna in soils, gravel, and loess (up to two meters in depth) on bedrock known to harbor fissure networks. The study aimed to describe the vertical distribution of spiders in these unique subterranean ecosystems and to describe species' preferences for certain types of substrates (microhabitats).

Materials and methods

Study sites

The research was carried out at ten sites in Czechia (Table 1, Fig. 1). In one site, Suchomasty, we documented temperature fluctuations measured by dataloggers Tinytag Ultra2 every three hours at the maximum sampling depth and on the surface (Fig. 5).

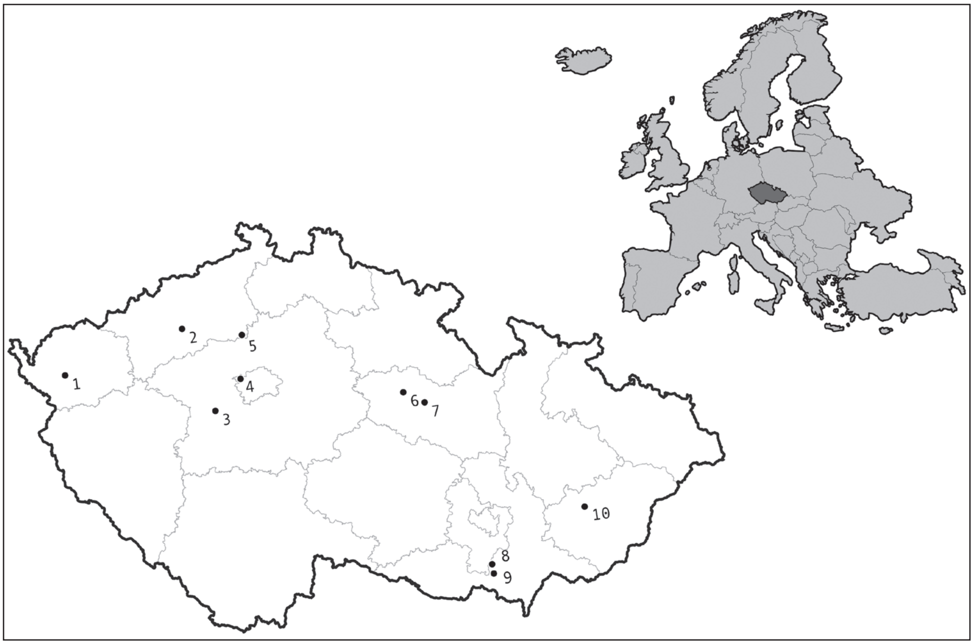

Figure 1. Locations of the study sites in Czechia. 1 – Kostelní Bříza, 2 – Raná, 3 – Suchomasty, 4 – Nebušice, 5 – Ctiněves, 6 – Hostovice, 7 – Mravín, 8 – Pouzdřany, 9 – Dolní Věstonice, 10 – Kurovice. For details, see Table 1.

Table 1. Characteristics of the study sites. n – number of pipe traps.

Site	Coordinates	Altitude [m a.s.l.]/average annual temperature	n/pipe length	Years	Substrate	Vegetation
Kostelní Bříza	50.1119°N, 12.6349°E	570/7 °C	2/110 and 80 cm	2017–2021	gravel accumulation from a metal mine	sparse *Pinus sylvestris* and *Betula pendula* wood
Raná	50.4067°N, 13.7450°E	250/9 °C	1/110 cm	2014–2016	stony rendzina soil and fissure network in sandy marlstone	steppe grassland
Suchomasty	49.9062°N, 14.0667°E	405/8 °C	3/200 cm	2015–2020	Stony brown soil on limestone bedrock	*Carpinion* forest
Nebušice	50.1019°N, 14.3050°E	350/9 °C	1/110 cm	2017–2021	stony brown soil and fissure network in sandy marlstone (Fig. 2B)	*Carpinion* forest
Ctiněves	50.3707°N, 14.3180°E	265/9°C	2/120 cm	2019–2021	thin ranker soil on sandy gravel	planted *Robinia* wood
Hostovice	50.0093°N, 15.8509°E	230/9°C	1/130 cm	2015–2018	Stony scree soil on claystone bedrock (Fig. 2C)	deciduous bush
Mravín	49.9445°N, 16.0516°E	315/9°C	2/120 cm	2013–2021	thin rendzina soil on sandy marl	oak forest
Pouzdřany	48.9429°N, 16.6430°E	290/10 °C	2/90 cm	2017–2021	Thin rendzina on loess	steppe grassland
Dolní Věstonice	48.8856°N, 16.6562°E	205/10 °C	1/90 cm	2019–2022	bare loess	deciduous bush
Kurovice	49.2736°N, 17.5249°E	305/9°C	2/100 cm	2013–2021	Thin brown soil on limestone bedrock	*Carpinion* forest

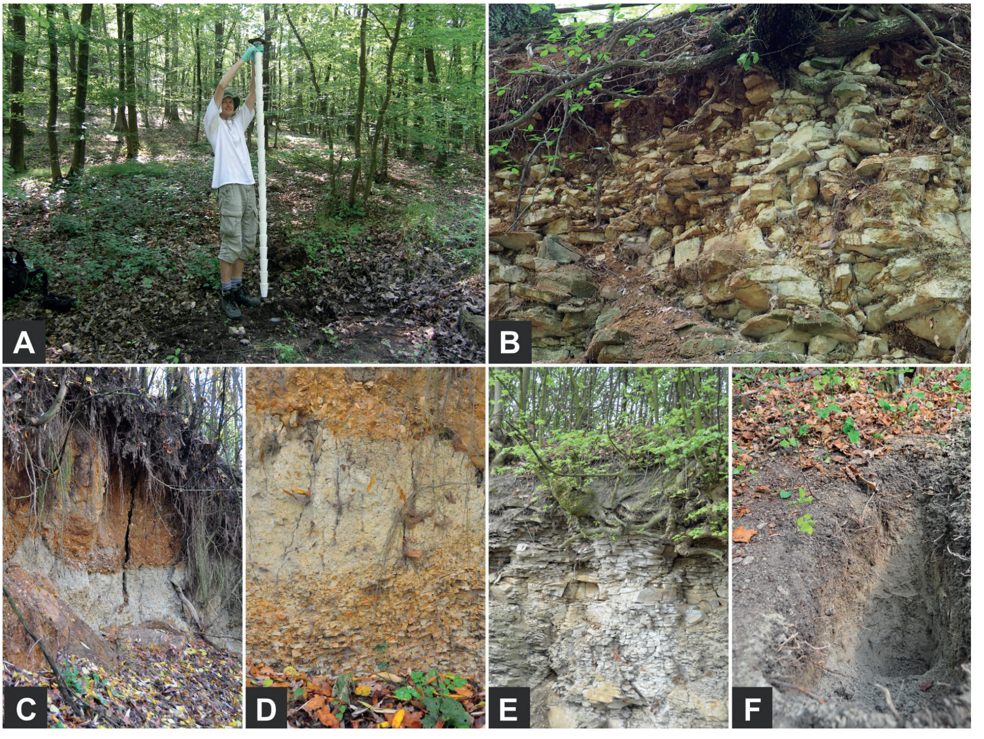

Figure 2. A a set of plastic cups taken from the pipe trap in the *Carpinus* forest on limestone near Suchomasty (photo M. Šafra); the soil profile is inhabited by *Porrhomma cambridgei*, see Fig. 3A **B** a fissure network in sandy marl near Nebušice, which was rich in *Porrhomma microcavense*, see Fig. 3D (the pipe trap was ten meters from this quarry wall) (photo M. Řezáč) **C, D** a scree soil near Hostovice, which was rich in *Porrhomma microps*, see Fig. 3E (photo J. Dolanský) **E** a fissure network in sandy marl near Mravín (the pipe trap was 200 meters from this profile) (photo J. Dolanský) **F** a hole for a pipe trap near Mravín in the soil, which was rich in *Iberina microphthalma* and *P. cambridgei*, see Fig. 3C (photo J. Dolanský).

Sampling

The plastic pipes (for lengths see Table 1) had an inner diameter of 7 cm and were perforated by oblique, 5 mm wide cuts. Plastic cups were mounted onto a threaded metal rod at 10 cm distances (for example, a 2-m long pipe contained 20 cups) (Fig. 2A) and contained a solution of 6% formaldehyde with few drops of detergent (for more details see Růžička and Dolanský 2016). We installed these traps in excavated holes or boreholes (a drilling machine was used in Suchomasty) and emptied them once a year. When we installed multiple pipes per site, each pipe was buried in a separate hole. Since traps were buried for long periods (between 2 and 8 years depending on the site), we expect the initial mixing of the soil layers to exert a minor role in the distribution of fauna.

Spider identification

Spiders were sorted and examined under an Olympus SZX12 stereomicroscope in 80% ethanol. Spider identification follows Nentwig et al. (2022), and nomenclature is in accordance with the World Spider Catalog (2022). The individuals were deposited at the institutions of the authors. Photographs were taken with an Olympus C-5060 wide-zoom digital camera mounted on an Olympus BX40 microscope. The images were processed using CombineZP image stacking software.

Relation to subterranean habitats

We classified the affinity of particular species to subterranean environments according to their vertical distribution. Further, we considered (1) morphological adaptations for subterranean life, (2) habitat, where they were found so far, (3) pattern of their distribution (common or rare on the surface habitats), (4) what environment they need for their lifestyle (see column "relation to the subterranean habitat – RSH" in Table 2). Morphological features, in particular depigmentation and a reduction in eye size, were recorded during identification, and the carapace size data were taken from the literature (Merrett et al. 1993; Weiss 1996; Růžička 2018, 2022; Šestáková et al. 2018; Nentwig et al. 2022). Data on spider occurrence in Czechia, habitat, and natural history were taken from a database of records of spiders in Czechia (Czech Arachnological Society 2022) and Buchar and Růžička (2002). The following categories were distinguished:

1 surface species occur only at the surface, on the ground, or in the vegetation. Their presence underground is accidental; they possess eyes of normal size and are normally pigmented.

2 detritus layer species live in detritus on the soil surface. They are rare in deeper soil layers. They possess eyes of normal size and are normally pigmented. Two special cases are within this category:

2B species with burrows in soil spend at least part of their lives in shallow burrows in soil; they possess eyes of normal size and are normally pigmented.

2H species requiring high air humidity require habitats with constantly high air humidity, regardless of whether the habitats are superficial or subterranean; they possess eyes of normal size and are normally pigmented.

3 soil species are more common deeper in humus soil than in detritus, but they appear on the soil surface relatively often; they are usually tiny and less pigmented, and their eyes are relatively smaller than those of their relatives. A special case is within this category:

3A soil ant nest species spend the majority of their lives in ant nests in the soil; they are less pigmented, and their eyes are relatively smaller than those of their surface relatives.

4 soil-bedrock species live in the basal mineral layers of soil on eroded bedrock surfaces (or in other spacious subterranean habitats) and appear on the soil surface very rarely; they are pale, and their eyes are significantly reduced; they can be larger with relatively long legs.

Data analyses

We used descriptive statistics [mean, standard error (SEM), and 95% confidence interval] to assess the species' preference for surface versus subterranean habitats, approximated via the depth of capture of each individual. In cases where the whole confidence interval was below a depth of 20 cm (the depth of the two uppermost cups in the trap, where the surface species can easily enter), we assumed the species to be more common below 20 cm depth than above it (the species shown in bold in Table 2). Data were handled in R, version 3.6.2 (R Core Team 2019).

Results

We captured 1179 adult spider specimens belonging to 76 species. In total, 17 species were found to be more common under the surface than on the surface (shown in bold in Table 2); singletons were not evaluated, but they are included in Table 2. Five species occurred preferentially below 70 cm (more than 15 specimens, Fig. 3). These were *Porrhomma cambridgei*, *Porrhomma microps*, *Porrhomma microcavense*, *Iberina microphthalma*, and *Centromerus* cf. *piccolo*. They possessed remarkably reduced eyes (Fig. 4). We called them "soil-bedrock species", as they probably occur in the interface between the soil and bedrock. In addition to these five species, we also considered *Pseudomaro aenigmaticus*, *Nesticus cellulanus*, *Jacksonella falconeri*, *Palliduphantes insignis*, *Centromerus* cf. *minutissimus* and *Theonoe minutissima* as belonging to this category. We further considered eight species to represent soil species (see the chapter Relation to subterranean habitats in MM for definitions of these categories: *Mioxena blanda*, *Porrhomma microphthalmum*, *Cicurina cicur*, *Tapinocyboides pygmaeus*, *Centromerus cavernarum*, *Centromerus serratus*, *Palliduphantes alutacius*, and *Palliduphantes pallidus*. We also recorded two myrmecophilous species occurring in ant nests in the soil, *Mastigusa arietina*, and *Syedra myrmicarum*. The remaining captured species were classified as detritus layer species (28) or as species with no relation to subterranean habitats (27).

Discussion

At least 21 species collected in our pipe traps exhibited morphological (eye reduction and depigmentation) and distributional (rare occurrence in surface habitats and regular presence in subterranean habitats) characteristics typical of subterranean fauna (categories 3 and 4 in Table 2). Of these, twelve species were more frequent below 20 cm than on the surface (marked bold in Table 2). The numbers of collected individuals in the remain-

Table 2. Vertical distribution of the collected spider species sorted according to the average depth from the deepest point toward the surface. Depth: mean ± SEM (95% confidence interval). The species that were significantly more common in deep subterranean habitats than close to the surface are shown in bold. n – number of specimens captured. Sites: Bří – Kostelní Bříza, Cti – Ctiněves, Hos – Hostovice, Kur – Kurovice, Mra – Mravín, Neb – Nebušice, Pou – Pouzdřany, Ran – Raná, Such – Suchomasty, Věs – Dolní Věstonice. RSH – relation to the subterranean habitat: 1 – surface species; 2 – detritus layer species (B – with burrows in the soil, H – requiring high air humidity); 3 – soil species (below the detritus layer, but found relatively often on the soil surface, A – ant nest species); 4 – soil-bedrock species (basal layers of the soil, very rarely found on the soil surface).

Species	Family	Depth [cm]	n	Geology	Site	RSH
Porrhomma cambridgei Merrett, 1994	Linyphiidae	100 ± 5.9 (89–112)	45	sandy marl, limestone	Mra, Such	4
Porrhomma microps (Roewer, 1931)	Linyphiidae	93 ± 2.0 (89–97)	156	sandy marl, limestone, claystone	Hos, Kur, Mra, Such	4
Porrhomma microcavense Wunderlich, 1990	Linyphiidae	86 ± 3.3 (80–93)	24	sandy marlstone	Neb	4
Mastigusa arietina (Thorell, 1871)	Dictynidae	80 ± 21.2 (38–122)	2	sandy marl, gravel heap	Bří, Mra	3A
Iberina microphthalma (Snazell & Duffey, 1980)	Hahniidae	78 ± 3.7 (70–85)	25	sandy marl	Mra	4
Centromerus cf. ***piccolo*** Weiss, 1996	Linyphiidae	75 ± 3.5 (68–82)	17	loess	Pou, Věs	4
Porrhomma campbelli F. O. Pickard-Cambridge, 1894	Linyphiidae	69 ± 2.3 (65–74)	12	gravel heap	Bří	2
Pseudomaro aenigmaticus Denis, 1966	Linyphiidae	65 ± 31.8 (3–127)	2	gravel heap	Bří	4
Walckenaeria capito (Westring, 1861)	Linyphiidae	63 ± 8.0 (47–78)	8	sandy marlstone, gravel heap	Bří, Ran	2
Mioxena blanda (Simon, 1884)	Linyphiidae	60 ± 23.0 (15–105)	3	sandy marl and marlstone, gravel	Cti, Mra, Ran	3
Nesticus cellulanus (Clerck, 1757)	Nesticidae	50 ± 0 (50)	1	claystone	Hos	4
Robertus neglectus (O. Pickard-Cambridge, 1871)	Theridiidae	50 ± 11.5 (27–73)	3	limestone	Such	2
Porrhomma microphthalmum (O. Pickard-Cambridge, 1871)	Linyphiidae	43 ± 9.0 (26–61)	3	sandy marl, limestone, claystone	Hos, Mra, Such	3
Cicurina cicur (Fabricius, 1793)	Dictynidae	42 ± 1.5 (39–45)	285	loess, sandy marl, marlstone, limestone, claystone, gravel heap	Bří, Hos, Kur, Mra, Neb, Ran, Such, Věs	3
Harpactea lepida (C. L. Koch, 1838)	Dysderidae	40 ± 5.4 (29–51)	7	limestone, gravel heap	Bří, Such	2H
Centromerus cavernarum (L. Koch, 1872)	Linyphiidae	40 ± 14.1 (12–68)	2	gravel heap	Bří	3
Jacksonella falconeri (Jackson, 1908)	Linyphiidae	40 ± 0 (40)	1	gravel heap	Bří	4
Coelotes terrestris (Wider, 1834)	Agelenidae	40 ± 0 (40)	1	sandy marl	Mra	2B
Micrargus herbigradus (Blackwall, 1854)	Linyphiidae	39 ± 3.8 (31–46)	25	sandy marl and marlstone, limestone, gravel heap	Bří, Kur, Mra, Neb	2
Palliduphantes insignis (O. Pickard-Cambridge, 1913)	Linyphiidae	35 ± 4.0 (27–43)	31	loess	Pou, Věs	4
Syedra myrmicarum (Kulczyński, 1882)	Linyphiidae	35 ± 3.5 (28–42)	2	sandy marl	Mra	3A
Palliduphantes alutacius (Simon, 1884)	Linyphiidae	34 ± 2.2 (30–38)	83	loess, sandy marl, claystone, limestone	Hos, Kur, Mra, Věs	3
Palliduphantes pallidus (O. Pickard-Cambridge, 1871)	Linyphiidae	33 ± 3.9 (26–41)	35	sandy marlstone, gravel heap	Bří, Neb, Ran	3
Centromerus cf. *minutissimus* Merrett & Powel, 1993	Linyphiidae	30 ± 0 (30)	1	gravel heap	Bří	4

Species	Family	Depth [cm]	n	Geology	Site	RSH
Metopobactrus prominulus (O. Pickard-Cambridge, 1873)	Linyphiidae	30 ± 0 (30)	1	gravel heap	Bří	2
Walckenaeria dysderoides (Wider, 1834)	Linyphiidae	30 ± 6.2 (18–42)	8	loess, limestone	Kur, Such, Věs	2
Walckenaeria obtusa Blackwall, 1836	Linyphiidae	30 ± 0 (30)	1	limestone	Kur	2
Robertus arundineti (O. Pickard-Cambridge, 1871)	Theridiidae	30 ± 0 (30)	1	limestone	Such	2
Piratula uliginosa (Thorell, 1856)	Lycosidae	30 ± 7.1 (16–44)	2	gravel heap	Bří	1
Clubiona terrestris Westring, 1851	Clubionidae	30 ± 7.1 (16–44)	2	sandy marl, claystone	Hos, Mra	1
Robertus lividus (Blackwall, 1836)	Theridiidae	26 ± 3.2 (19–32)	18	loess, limestone	Kur, Pou, Věs	2
Agyneta rurestris (C. L. Koch, 1836)	Linyphiidae	25 ± 3.5 (18–32)	2	limestone, gravel heap	Bří, Such	1
Histopona torpida (C. L. Koch, 1837)	Agelenidae	25 ± 10.6 (4–46)	2	sandy marl	Mra	2H
Hahnia pusilla C. L. Koch, 1841	Hahniidae	24 ± 2.1 (20–28)	16	sandy marl, limestone, gravel heap	Bří, Kur, Mra	2
Harpactea rubicunda (C. L. Koch, 1838)	Dysderidae	23 ± 2.7 (18–29)	6	sandy marl and marlstone, limestone	Mra, Neb, Such	2
Theonoe minutissima (O. Pickard-Cambridge, 1879)	Theridiidae	23 ± 2.7 (18–29)	6	gravel heap	Bří	4
Dysdera cechica Řezáč, 2018	Dysderidae	23 ± 3.7 (16–30)	10	loess, limestone	Kur, Pou, Věs	2
Diplostyla concolor (Wider, 1834)	Linyphiidae	22 ± 1.4 (20–25)	102	claystone, sandy marlstone, limestone	Hos, Kur, Neb, Ran	2
Dysdera erythrina (Walckenaer, 1802)	Dysderidae	20 ± 2.2 (16–24)	11	limestone, gravel heap	Bří, Such	2
Episinus truncatus Latreille, 1809	Theridiidae	20 ± 7.7 (5–35)	3	loess	Pou	1
Mermessus trilobatus (Emerton, 1882)	Linyphiidae	20 ± 0 (20)	1	claystone	Hos	1
Tapinocyba insecta (L. Koch, 1869)	Linyphiidae	20 ± 0 (20)	1	gravel	Cti	2
Tapinocyboides pygmaeus (Menge, 1869)	Linyphiidae	20 ± 0 (20)	1	gravel heap	Bří	3
Amaurobius jugorum L. Koch, 1868	Amaurobiidae	20 ± 0 (20)	1	sandy marl	Mra	2B
Trochosa ruricola (De Geer, 1778)	Lycosidae	20 ± 7.7 (5–35)	3	sandy marlstone	Ran	2B
Phrurolithus festivus (C. L. Koch, 1835)	Phrurolithidae	18 ± 1.7 (15–22)	31	loess, sandy marl and marlstone, limestone, gravel heap, gravel	Bří, Cti, Mra, Neb, Ran, Such, Věs	1
Centromerus sylvaticus (Blackwall, 1841)	Linyphiidae	17 ± 1.8 (13–20)	13	loess, limestone, sandy marlstone, gravel heap	Bří, Neb, Ran, Such, Věs	2
Trachyzelotes pedestris (C. L. Koch, 1837)	Gnaphosidae	17 ± 1.2 (14–19)	18	loess, sandy marlstone, limestone, gravel	Cti, Kur, Ran, Věs	1
Inermocoelotes inermis (L. Koch, 1855)	Agelenidae	16 ± 3.2 (10–22)	5	limestone, gravel heap	Bří, Kur	2B
Haplodrassus silvestris (Blackwall, 1833)	Gnaphosidae	15 ± 3.5 (8–22)	2	limestone	Kur	1
Centromerus serratus (O. Pickard-Cambridge, 1875)	Linyphiidae	14 ± 2.1 (10–18)	5	sandy marl	Mra	3
Agroeca cuprea Menge, 1873	Liocranidae	13 ± 1.4 (8–32)	3	sandy marlstone, loess	Pou, Ran	1
Apostenus fuscus Westring, 1851	Liocranidae	13 ± 0.7 (11–14)	31	limestone	Kur	1
Tenuiphantes flavipes (Blackwall, 1854)	Linyphiidae	11 ± 0.5 (10–12)	11	loess, limestone	Kur, Such, Věs	1
Euryopis flavomaculata (C. L. Koch, 1836)	Theridiidae	10 ± 0 (10)	2	limestone	Kur	2

Species	Family	Depth [cm]	n	Geology	Site	RSH
Agyneta saxatilis (Blackwall, 1844)	Linyphiidae	10 ± 0 (10)	1	loess	Pou	1
Linyphia hortensis Sundevall, 1830	Linyphiidae	10 ± 0 (10)	1	sandy marl	Mra	1
Micrargus subaequalis (Westring, 1851)	Linyphiidae	10 ± 0 (10)	1	sandy marlstone	Ran	2
Oedothorax retusus (Westring, 1851)	Linyphiidae	10 ± 0 (10)	1	limestone	Kur	2
Pelecopsis radicicola (L. Koch, 1872)	Linyphiidae	10 ± 0 (10)	1	gravel heap	Bří	2
Aulonia albimana (Walckenaer, 1805)	Lycosidae	10 ± 0 (10)	1	limestone	Kur	2B
Pardosa lugubris (Walckenaer, 1802)	Lycosidae	10 ± 0 (10)	45	sandy marlstone, limestone	Kur, Neb	1
Pardosa riparia (C. L. Koch, 1833)	Lycosidae	10 ± 0 (10)	5	sandy marlstone	Ran	1
Trochosa terricola Thorell, 1856	Lycosidae	10 ± 0 (10)	2	limestone	Such	2B
Zora spinimana (Sundevall, 1833)	Miturgidae	10 ± 0 (10)	1	limestone	Kur	1
Agroeca brunnea (Blackwall, 1833)	Liocranidae	10 ± 0 (10)	1	limestone	Kur	1
Phrurolithus pullatus (C. L. Koch, 1835)	Phrurolithidae	10 ± 0 (10)	1	loess	Pou	1
Zodarion germanicum (C. L. Koch, 1837)	Zodariidae	10 ± 0 (10)	5	limestone	Kur	1
Drassyllus praeficus (L. Koch, 1866)	Gnaphosidae	10 ± 0 (10)	1	loess	Pou	1
Drassyllus pumilus (C. L. Koch, 1839)	Gnaphosidae	10 ± 0 (10)	4	loess	Pou	1
Haplodrassus umbratilis (L. Koch, 1866)	Gnaphosidae	10 ± 0 (10)	3	gravel heap	Bří	1
Zelotes apricorum (L. Koch, 1876)	Gnaphosidae	10 ± 0 (10)	8	limestone	Kur	1
Ozyptila praticola (C. L. Koch, 1837)	Thomisidae	10 ± 0 (10)	1	limestone	Kur	1
Ozyptila trux (Blackwall, 1846)	Thomisidae	10 ± 0 (10)	1	loess	Pou	1
Xysticus luctator L. Koch, 1870	Thomisidae	10 ± 0 (10)	1	limestone	Kur	1
Euophrys frontalis (Walckenaer, 1802)	Salticidae	10 ± 0 (10)	2	limestone	Kur	1

ing nine species were too low to demonstrate the same preference. On the other hand, five other species, *Porrhomma campbelli*, *Walckenaeria capito*, *Robertus neglectus*, *Harpactea lepida*, and *Micrargus herbigradus*, did not exhibit morphological or distributional features (they are common in surface habitats) characteristic of subterranean fauna but were more frequent below 20 cm than on the surface (also marked bold in Table 2). However, this result is based on low specimen numbers. The remaining species did not have any affinity for the subterranean environment; they usually occur on the soil surface or in detritus. These species probably crawled down the substrate along the pipe where the soil became loose due to digging. Such vertical migration could have happened during autumn and winter months when even the surface species search for suitable spots for overwintering.

The main subterranean spider groups

The captured subterranean species belonged to the families Linyphiidae, Hahniidae, Theridiidae, and Nesticidae, with Linyphiidae having the greatest abundance and species richness. Three genera dominate in the Central European shallow subterranean habitats: *Centromerus*, *Palliduphantes*, and especially *Porrhomma*. At two study sites, Mravín and Suchomasty, *Porrhomma cambridgei* and *Porrhomma microps* were found to occur syntopically. In both cases, the smaller *P. cambridgei* was found deeper than the larger *P. microps* (Fig. 3A, C). So far, *Porrhomma campbelli* has been known from the moss in wet habitats, such as peat bogs, spruce forests, and brook and pond margins (Růžička 2018). Here, we present the first finding of this species from subterranean habitats in Czechia.

The genus *Centromerus* was represented by four species, two of which, *C.* cf. *piccolo* and *C.* cf. *minutissimus*, were new to Czechia. We captured 17 specimens of *Centromerus* cf. *piccolo* at depths of 50–90 cm in loess accumulations in south Moravia. Previously, *Centromerus piccolo* had been known only from Germany (Weiss 1996; Nentwig et al. 2022). As the genitalic morphology of our specimens slightly differs from the illustrated type specimens of *C. piccolo* (Weiss 1996), we can not exclude the possibility that this is a closely related new species. In the artificial gravel accumulation in western Bohemia, we found two *Centromerus* females

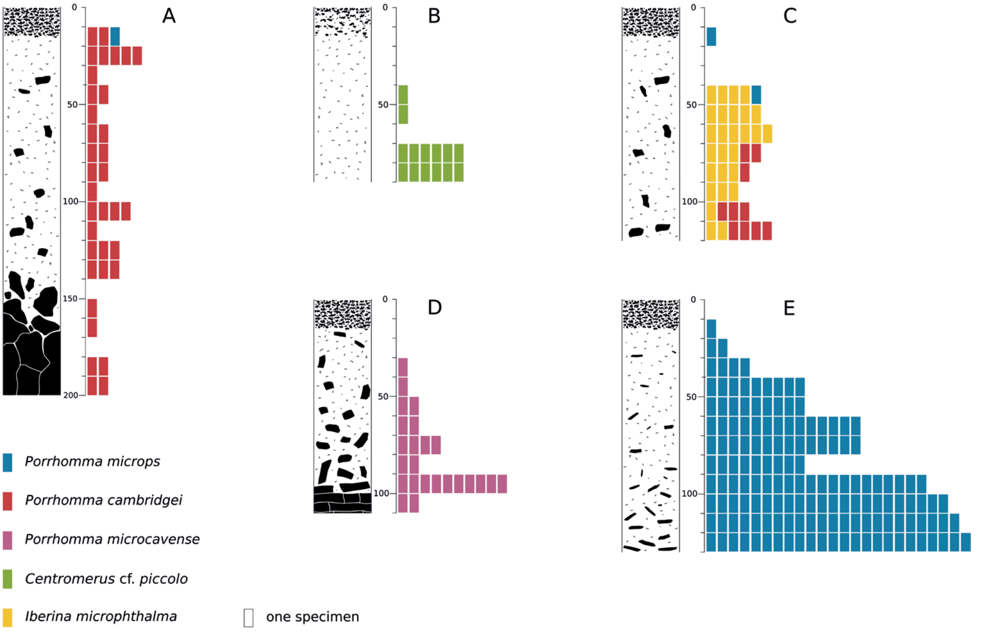

Figure 3. Panels show soil profiles and the depth-dependent occurrence of microphthalmic spider species (depths in cm) **A** Suchomasty, soil on limestone **B** Věstonice, loess **C** Mravín, stony soil above sandy marl **D** Nebušice, stony soil, and fissure network in sandy marl **E** Hostovice, stony soil above claystone.

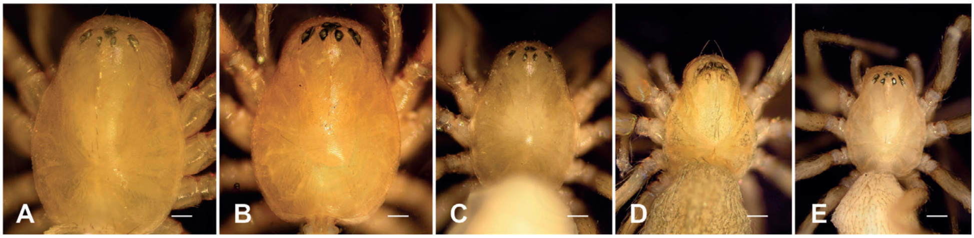

Figure 4. Carapaces of female spider species found deep in the Czech shallow subterranean habitats **A** *Porrhomma microps* from Hostovice **B** *P. microcavense* from Nebušice **C** *P. cambridgei* from Suchomasty **D** *Iberina microphthalma* from Mravín **E** *Centromerus* cf. *piccolo* from Dolní Věstonice. Scale bars: 0.1 mm (photos V. Růžička).

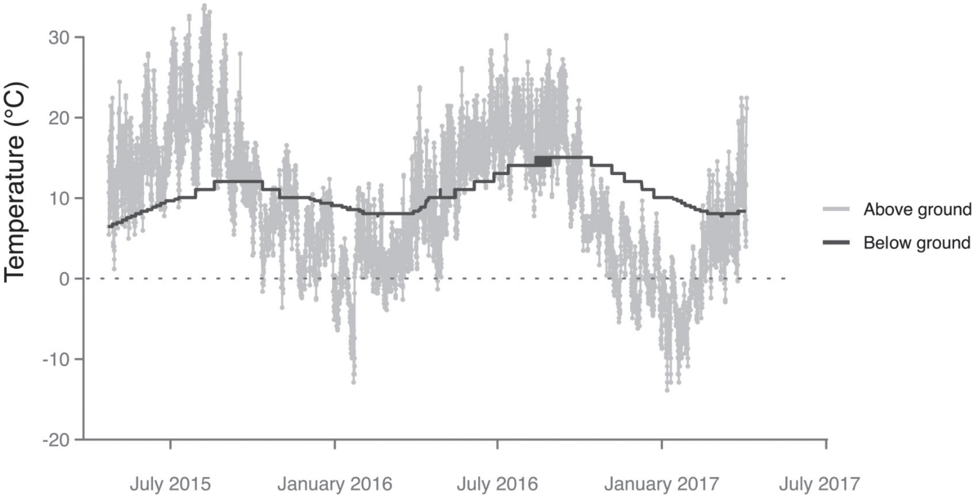

Figure 5. Temperature fluctuations in shallow subterranean habitats (below ground) were minor compared to temperature fluctuations on the surface (above ground). Data were collected from the study site at Suchomasty, between 23 April 2015 to 15 April 2017, taking one measurement every three hours. Above ground: 2.5 m above the ground, in shade. Below ground: at 2 m depth.

that are not described in the literature. Based on somatic characters (including small body size, eye reduction, and depigmentation) and the fact that the only Central European *Centromerus* species with unknown females was *Centromerus minutissimus*, which were known from England, Germany (the site in southern Saxony-Anhalt is only 200 km northwest of our site), south France and Spain (Merrett et al. 1993; Cárdenas and Barrientos 2011; Nentwig et al. 2022, GBIF – www.gbif.org, INPN – www.inpn.mnhn.fr), we preliminarily determined these individuals to be *Centromerus* cf. *minutissimus*.

We captured three species of the genus *Palliduphantes*. *Palliduphantes insignis* has been found in Czechia very rarely, only on the steppes on rocks or loess in warm areas

(Buchar and Růžička 2002). We found 31 specimens at a depth of approximately 30 cm in loess. Thus, its preferred microhabitats are probably micro spaces in soil and fissures in bedrock. *Palliduphantes pallidus* and *Palliduphantes alutacius* sensu Miller and Obrtel (1975) are closely related taxa that are not morphologically clearly separated in Czechia. Instead, there are mainly typical *Palliduphantes pallidus* in the western part of Czechia, while in the eastern part typical *Palliduphantes alutacius* occurs, and morphologically intermediate forms can be found between. This pattern probably demonstrates the introgression of these two taxa in this territory. These species are among the most common subterranean species in Czechia and are also often found at the surface.

Concerning the remaining subterranean linyphiid spiders, we found four species of the subfamily Erigoninae: *Jacksonella falconeri*, *Mioxena blanda*, *Pseudomaro aenigmaticus*, and *Tapinocyboides pygmaeus*. Singletons of these species have rarely been found on the surface, usually in open xerothermic habitats (Czech Arachnological Society 2022). Our data show that the main microhabitats of these species are interspaces in various substrata.

The subterranean Hahniidae spiders were represented by three species. European records of *Iberina microphthalma* were summarized by Růžička and Dolanský (2016). Furthermore, in Czechia, this species was collected in the substratum on claystone, sandy marlstone, or sandy marl (Růžička 2022). We found 17 specimens in the soil on sandy marl at depths of 40–120 cm (Fig. 3C). *Cicurina cicur* is common under stones, in leaf litter, and decaying wood in forests, as well as in shallow subterranean habitats (Buchar and Růžička 2002; Růžička and Dolanský 2016). Because of its morphological appearance – depigmentation and small eyes – it is expected for this species to spend most of its life in the subterranean environment, especially in southern Europe (Mammola et al. 2022). Indeed, we found it to be dominant in the studied shallow subterranean habitats.

Nesticus cellulanus is the only representative of Nesticidae, the spiders mainly occurring in subterranean environments (Mammola et al. 2022). As this species is relatively large, it mainly occurs in caves, scree slopes, or man-made subterranean spaces, whereas it is rare in interspaces in the substratum.

The family Theridiidae was represented by *Theonoe minutissima* and some species of the genus *Robertus*. *Theonoe minutissima* regularly occurs in scree slopes, we found it only inside artificial gravel accumulation. *Robertus* species recorded during our study are common in detritus.

Myrmecophilous species

At two sites, Kostelní Bříza and Mravín, we captured the relatively large depigmented hahniid spider *Mastigusa arietina*, which is known to live in subterranean ant nests (Nentwig et al. 2022). At the Mravín site, it was found with another myrmecophilous spider, *Syedra myrmicarum*. The dominant co-occurring ant species was *Lasius flavus* (Fabricius, 1782).

Troglomorphism versus edaphomorphism

The body size and relative length of appendages of subterranean species may depend on the size of the spaces they inhabit (Pipan and Culver 2017). Predictably, an elongation of appendages is an adaptation to life in relatively large subterranean spaces, typically caves (troglomorphism, Zacharda 1979). In contrast, a diminished body size represents an adaptation to life in relatively small, narrow subterranean spaces, typically of soils (edaphomorphism, Zacharda 1979). Altogether, the species collected during this study cover the continuum from troglomorphic to edaphomorphic species. For example, relatively large, long-legged *Porrhomma microps* (carapace width 0.84 mm) and *P. microcavense* (carapace width 0.75 mm) represent typical troglomorphic species. They are characteristic of the interface between soil and sandy marlstone that harbors relatively large interspaces (Fig. 2B). Because of harsh climatic conditions during Quaternary glaciations, Central Europe lacks large troglomorphic spiders, known, for example, from Mediterranean caves (Culver et al. 2006). The most troglomorphic species of this region are still small enough to also occur in soil (Růžička 1999).

The edaphomorphic species collected during this study were tiny *Centromerus* (*C.* cf. *piccolo* and *C.* cf. *minutissimus*), *Porrhomma cambridgei*, *Iberina microphthalma*, and tiny Erigoninae linyphiids, all of which have carapace widths less than 0.6 mm. They probably live in narrow interspaces in soil or loess; however, they also occur in deep caves (Růžička and Buchar 2008; Růžička 2018, 2022).

Preferences for bedrock and climate

Different shallow subterranean habitats presumably provide microhabitats that are preferred by different species. Indeed, some subterranean species collected in the soil-bedrock interface seem to exhibit a preference for specific depths and substrates. For example, *Centromerus* cf. *piccolo* is characteristic of loess, and *Porrhomma microcavense* and *Iberina microphthalma* are characteristic of sandy marlstone. The species that occur in the surface layer of the soil seem not to express such preferences (for example, *Centromerus serratus*, *C. cavernarum*, *Mioxena blanda*, *Palliduphantes* spp., *Porrhomma microphthalmum*, *P. microps*, *Tapinocyboides pygmaeus*, *Cicurina cicur*).

The fauna of shallow subterranean habitats seems to be richer in warm areas than in cold ones. The species *Centromerus* cf. *piccolo*, *Mioxena blanda*, *Palliduphantes insignis*, *Porrhomma cambridgei*, and *Iberina microphthalma* are restricted to the warmest regions of Czechia. Additionally, vegetation can be expected to modify the temperature regime in the soil. A large number of the soil species found during this study live in substrates that occur in partly open xerothermic habitats (for example, *Centromerus* cf. *piccolo*, *Palliduphantes insignis*, *Tapinocyboides pygmaeus*, and *Iberina microphthalma*). On the other hand, some species live in habitats that maintain stable humidity and temperature, such as scree forests (e.g., *Centromerus cavernarum*, *Porrhomma campbelli*, *Theonoe minutissima*).

Dispersal

Interestingly, the only artificial habitat that was studied, an almost hundred-year-old gravel heap from a polymetallic ore mine near Kostelní Bříza, harbors relatively rich subterranean fauna. Several species with relatively small eyes, in particular *Jacksonella* *falconeri*, *Pseudomaro aenigmaticus*, *Tapinocyboides pygmaeus*, *Centromerus cavernarum*, *Centromerus* cf. *minutissimus*, *Palliduphantes pallidus*, *Porrhomma campbelli*, *Theonoe minutissima*, *Cicurina cicur*, and *Mastigusa arietina* were found here. Thus, at least these species are able to colonize new isolated sites, probably by ballooning, and they cannot be considered relics like the endemic fauna in limestone caves in the Mediterranean. For *Pseudomaro aenigmaticus*, this ability was confirmed by the capture of specimens in aeroplankton at 12 m height (Blick and Kreuels 2002).

Conclusion

Our study demonstrates that underexplored shallow subterranean habitats in Central Europe harbor rich subterranean spider fauna. Some of these species were considered very rare in the past. However, we came to the same conclusion as Polenec (1970): these species are, in fact, quite abundant in these hardly accessible and largely overlooked habitats.

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